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CLINICAL RESEARCH ARTICLE

Growth, Bone Health & Ambulatory Status of Boys with DMD Treated With Daily vs. Intermittent Oral Glucocorticoid Regimen

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Abstract

Oral glucocorticoids (GC) preserve muscle strength and prolong walking in boys with Duchenne muscular dystrophy (DMD). Although vertebral fractures have been reported in boys taking GC, fracture rates for different GC regimes have not been investigated. The aim of this pragmatic longitudinal study was to compare growth, body mass, bone mineral density (BMD), vertebral fractures (VF) and ambulatory status in boys with DMD on daily (DAILY) or intermittent (INTERMITTENT), oral GC regimens.

A convenience sample of 50 DMD boys from two centres was included in the study; 25 boys each were on the DAILY or INTERMITTENT regimen. Size adjusted lumbar spine BMD (LS BMAD), total body less head BMD (TBLH), by DXA and distal forearm bone densities by pQCT, GC exposure, VF assessment and ambulatory status were analysed at three time points; baseline, 1 and 2 years.

At baseline, there were no differences in age, GC duration or any bone parameters. However, DAILY boys were shorter (height SDS DAILY= -1.4(0.9); INTERMITTENT= -0.8(1.0), p=0.04) with higher BMI (BMI SDS DAILY= 1.5(0.9); INTERMITTENT= 0.8(1.0), p=0.01). Over 2 years, DAILY boys got progressively shorter (delta height SDS DAILY= -0.9(1.1); INTERMITTENT= +0.1(0.6), p<0.001). At their 2 year assessment, 5 DAILY and 10 INTERMITTENT boys were non-ambulant. DAILY boys had more VFs than INTERMITTENT boys (10 versus 2; $\chi 2$ p = 0.008). BMAD SDS remained unchanged between groups. TBLH and radius BMD declined significantly but the rate of loss was not different.

In conclusion, there was a trend for more boys on daily GCs to remain ambulant but at the cost of more VFs, greater adiposity and markedly diminished growth. In contrast, boys on intermittent GCs had fewer vertebral fractures but there was a trend for more boys to loose independent ambulation.

Key words: Glucocorticoids, Muscular dystrophy, vertebral fracture, ambulation, bone density, growth

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, which affects 1 in 3600–6000 live male births [1]. DMD is caused by loss of function mutations in the *dystrophin* gene, which encodes the dystrophin protein in muscle. Dystrophin deficiency results in necrosis of myofibres, which in turn results in progressive deterioration of muscle function. The disease manifests usually before 3 years of age with proximal muscle weakness and the inability to run and jump as their peers. As the disease progresses, rising from the floor becomes difficult and eventually independent ambulation is lost.

Currently there is no cure for DMD but the quality of life of patients can be improved by medical treatment and supportive care. Long term treatment with oral glucocorticoids (GCs) slows down deterioration of skeletal muscle function, prolonging ambulation by 2 to 5 years [2]. Moderate quality evidence from RCTs demonstrates that GC treatment improves muscle function for about 12 months, and muscle strength for up to two years [3]. Treatment with GCs along with improved cardiorespiratory and orthopaedic supportive care, have led to improved survival of males with DMD. The current standard of care is to treat with prednisolone 0.75 mg/kg/day or deflazacort 0.9mg/kg/day; this treatment is initiated at the plateau of motor abilities between 4 and 6 years [1]. After loss of ambulation, steroid treatment may be continued, usually without further weight-related dose increase, or discontinued if side-effect burden is intolerable. Within the UK, GC treatment is given either daily or intermittently (10 days on & 10 days off) [4].

Long-term GC treatment in DMD boys is associated with obesity, short stature, pubertal delay, and an increased risk of long bone and vertebral fractures (VFs) [5]. Fractures are associated with low bone mineral density (BMD) [6-12] and mainly caused by progressive muscle weakness and GC use[13]. Furthermore, GC-related growth deceleration and pubertal delay might also contribute to bone fragility in DMD boys [5]. Long bone fractures may precipitate permanent loss of mobility [8]. VFs may be asymptomatic or associated with severe localised back pain. The role of chronic GC therapy in the causation of VFs in DMD is emphasized by their absence in boys who are GC naïve [10]. Although VF have previously been reported in DMD boys taking GCs [7, 9-12, 14], fracture rate for different GC regimes has not been investigated.

Clinical observation suggests that DMD boys on continuous GC regimes may be more prone to VFs compared to those on intermittent regimes. However, to the best of our knowledge bone health outcomes have not been compared in DMD boys on different GC regimes. Thus, the aim of this study was to compare longitudinal growth, body mass, BMD, VFs and ambulatory status in boys with DMD on daily or intermittent, oral GC regimens.

2. Materials and Methods

This was a pragmatic study to compare the two different GC regimes. The majority of children from Manchester are treated using the continuous GC regime (DAILY), whilst the majority of children from Birmingham are treated using the intermittent 10 days on-10 days off regime (INTERMITTENT). The decision to commence GC therapy was made by the local neuromuscular consultant according to the guidelines used by the NorthStar network [4].

2.1. Subjects

A retrospective convenience sample of boys with established DMD was included, managed in two UK centres between 2006 and 2014. At both centres, boys were excluded if they were not on the daily or intermittent GC regime, for e.g. alternate day or weekend only regimes. Additionally, boys were excluded if they had had exposure to oral or intravenous bisphosphonate treatment at baseline bone assessment. At Manchester Children's Hospital, 25 boys were selected who had undergone annual "bone health" assessments on at least 3 consecutive time points irrespective of their age (i.e. had at least 2 years follow up). The same number of children was then identified from Birmingham Children's Hospital DMD bone database in order to match as far as possible with those in Manchester, by age and imaging modality. At both centres, bisphosphonate treatments were initiated when there was evidence of a VF and associated pain. This information was extracted from the boys' medical records.

At both centres, boys are routinely given advice to optimise their dietary calcium intake, preferably from low fat dairy products; they also receive vitamin D supplements and 25-hydroxy vitamin D levels are monitored annually.

2.2. Ethics

Since this project was undertaken as a service / therapy evaluation between two UK centres, it did not require ethical approval [15].

2.3. Cumulative Glucocorticoid Exposure

Cumulative GC exposure was calculated using information recorded in the subjects' medical records.

2.4. Anthropometric measurements

Height and weight were measured and body mass index calculated as weight/height^2 (kg/m^2). Once the child was unable to stand, arm span or supine length were used as surrogates for height. Height, weight and BMI measurements were transformed to standard deviation scores using the 1990 British growth reference data [16]. At both centres, anthropometric and bone assessments were repeated approximately annually.

2.5. Ambulation Status

At each bone imaging time-point, the subject's mobility status was recorded, categorised as independently ambulant or requiring wheelchair assistance.

2.6. Bone Health Assessments

2.6.1. Vertebral Fracture Assessment (VFA)

The VFA was undertaken, at each measurement point, using all available spine imaging (e.g. CT scout views, anterior and lateral whole spine radiographs and Dual-Energy X-ray Absorptiometry (DXA). Until recently vertebral fracture assessment, (VFA) by DXA was considered inferior to plain X-rays for the diagnosis of vertebral fracture. However, with the development of new higher-resolution scanners, VFA by DXA is proving to be an attractive imaging tool for children. Compared to lateral radiographs, it affords the child a significant (approximately threefold) reduction in radiation dose and is available at the time of routine DXA scanning [17, 18].

Images from both centres were jointly reviewed by 2 experienced investigators (JEA & NJC) to arrive at a consensus on fracture identification. Fractures were defined if there was ≥ 25% loss in height of anterior, mid or posterior vertebral body, in relation to the adjacent unaffected vertebrae [19]. This

threshold was based on a UK based survey which suggested that ≥ 25% loss in vertebral height was the most likely level to prompt bisphosphonate treatment [20]. In addition, the identification of VFs with less than 25% vertebral height loss is unreliable both by plain radiography and DXA based VFA [17, 18].

2.6.2. Long bone Fractures

Data on long bone fractures was collected from review of hospital records and confirmed where possible from radiological review.

2.6.3. Dual-Energy X-ray Absorptiometry (DXA)

DXA scans of the lumbar spine (L1-4) and total body were performed on either a GE Lunar iDXA™ or Prodigy (GE Lunar Corp. Madison, WI, USA) (Birmingham) or a Hologic Discovery (QDR 4500 Discovery, Hologic Inc. Bedford, MA) (Manchester) scanner, according to standard protocol. Results are presented as lumbar spine bone mineral apparent density [L1-L4 BMAD (g/cm^3)] [21] and total body less head BMD [TBLH BMD (g/cm^2)]. Age, gender and machine specific Z-scores were calculated using UK reference data [22].

2.6.4. Peripheral quantitative computed tomography (pQCT)

At both sites, pQCT scans were acquired at the distal radius (4% of radial length) of the non-dominant forearm using a Stratec XCT-2000 scanner (Stratec, Pforzheim, Germany). Outcome variables included trabecular and total volumetric BMD (g/cm^3). Age- and gender-specific Z-scores were calculated using the manufacturer's reference data [23].

2.7. Statistical Analysis

Analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp, Armonk, NY). Parameter differences from baseline to follow-up were evaluated using General Linear Model Repeated Measures analyses, with time as the within-subject factor and GC regime (DAILY or INTERMITTENT) as the between-subjects factor. If the sphericity assumption was not met, the Huynh-Feldt correction was applied. A level of $p<0.05$ was used to denote statistical significance. Results are presented as mean and standard deviation unless otherwise stated.

3. Results

3.1. Baseline Characteristics

By design, subjects from both GC regimes did not differ in age (mean (SD) 8.3 (2.4), age of GC therapy initiation (6.3 (1.9) years) or duration of GCs 2.0 (1.9) years). However, boys on the DAILY regime had greater cumulative GC exposure and were significantly shorter (height SDS -1.4 (1.0) vs. -0.8 (1.0) p=0.04) with greater BMI (BMI SDS 1.5 (0.9) vs. 0.8 (1.0) p=0.01). In contrast, there were no differences in any of the measured bone density parameters between treatment regimens whether we included or excluded (data not shown) boys who subsequently went on to receive bisphosphonate therapy (Table 1). One boy from the DAILY group and 3 boys from the INTERMITTENT group had suffered a long bone fracture. In contrast, 1 of the DAILY boys had radiological evidence of a vertebral fracture, whereas none of the INTERMITTENT boys had evidence of vertebral fracture.

3.2. Follow-up Data

3.2.1. Growth and body mass

After 2.5 (0.9) years, height SDS decreased significantly in the DAILY boys but remained constant for the INTERMITTENT boys (delta height SDS -0.9 (1.1) vs. +0.1 (0.6); p<0.001). Weight SDS increased significantly in both the DAILY boys and INTERMITTENT boys (delta weight SDS +0.3 (0.8) vs. +0.7 (0.7); p=0.05). In contrast, BMI SDS increased at the same rate for both DAILY and INTERMITTENT boys (mean delta BMI SDS = +0.8 (0.8)). Consequently, BMI SDS for DAILY boys remained significantly higher at the final assessment (BMI SDS DAILY = +2.3 (0.7) vs. INTERMITTENT = +1.5 (1.0); p=0.001).

3.2.2. Ambulation, fractures and bone density

There was a non-significant trend for more boys in the INTERMITTENT regime to be non-ambulant. At their final assessment, mean age 10.7 (2.7) years, 10 boys on the INTERMITTENT regime (40%) and 5 in the DAILY group (20%) were non ambulant (Figure 1a).

During follow-up, one DAILY boy sustained a minimally displaced metaphyseal fracture of the distal tibia. None of the INTERMITTENT boys sustained any long bone fractures. Over the same period, 10 (40%) DAILY and 2 (8%) INTERMITTENT boys suffered VFs (χ^2 =7.018; p =0.008) (Figure1b). Six of the 10 DAILY boys with symptomatic VF prior to the 2 year assessment were commenced on intravenous bisphosphonate therapy. As such, bone density data from these 6 boys were excluded from follow-up comparisons.

LS BMAD Z-scores did not change over time and did not differ between the treatment groups. In contrast, Z-scores for TBLH BMD, TotBMD and TrabBMD at the 4% distal radial site decreased significantly ($p<0.05$) with time. However, there was no statistical interaction between GC regime and time for any of the bone parameters (Table 2).

Similar differences were observed combining GC regimes and grouping according to fracture. There were no significant differences in LS BMAD or TBLH BMD Z-scores between those that did or did not suffer a VF. However, TotBMD and TrabBMD Z-scores at the 4% distal radius were significantly lower in boys who fractured ($p<0.05$), and declined ($p<0.05$) over time in both groups (Table 3 & Figure 2b).

4. Discussion

This study found that boys on the DAILY GC regime suffered significantly more VFs than those on the INTERMITTENT GC regimen but more tended to be ambulant at the end of the observation period. DAILY boys were also shorter, grew less and had greater BMI.

Despite the striking differences in VFs there were no differences in BMAD between DAILY and INTERMITTENT GC regimes either at baseline or over the duration of follow-up. Similarly, in the whole group, there were also no differences in BMAD between boys with VFs and those without. Thus BMAD, in DMD boys treated with GC, does not appear to be a predictive marker of vertebral fracture. This supports observations in children with nephrotic syndrome [24] but contrasts findings from the STOPP study in children with acute lymphoblastic leukaemia, nephrotic syndrome and rheumatoid arthritis, where low areal BMD was a significant predictor of VFs [25, 26]. The lumbar spine BMAD may not be useful for differentiating boys with and without VFs in DMD.

Distal radial total and trabecular bone density measured by pQCT were not different between GC regimes at baseline. However, in contrast to BMAD, the pQCT density (g/cm^3) Z-scores decreased over time. We postulate that the differences between BMAD and pQCT distal radial densities may be due to the higher amount of trabecular bone in the pQCT measured distal radial parameters and the greater sensitivity of pQCT to detect trabecular changes. Adult studies indicate that GC have a predilection to affect the more metabolically active trabecular bone [27]. If this holds true for children, it would explain why reductions in trabecular rich sites as measured by pQCT are more sensitive to change than the lumbar spine.

It is generally assumed that BMAD reflects predominantly trabecular bone density of the spine; however, because DXA measures the whole projection, at the lumbar spine it invariably includes the cortical spinous processes. The combination of trabecular bone in the vertebral body with the cortical bone from the spinous processes may mask any changes within the trabecular compartment arising from GC and progressive immobility of the DMD boys. Although DXA remains the preferred method of bone health assessment in children [28], it is well known that areal bone mineral density, as measured by DXA, is significantly affected by bone size. Children with reduced stature and hence reduced bone size will have spuriously low areal bone mineral density [29]. In our group of boys the mean Z score for height, in both groups at the start of the assessment was reduced. However, boys on the DAILY regime were already significantly shorter at baseline and grew less than the intermittent group. To overcome the known pitfall of bone size in DXA imaging, we used the bone mineral apparent density (BMAD) to assess their bone changes [28]. However, even though BMAD reduces the size influence it is only an approximation for true volumetric bone density. As such, quantitative computed tomography (QCT) of the lumbar spine may be a more appropriate lumbar spine bone density technique to apply in children with DMD. We speculate that volumetric BMD by spine QCT may a better predictor of vertebral fracture. Longitudinal studies are needed to answer this question [30].

Our pragmatic study has a number of shortcomings. The data on ambulatory status was gathered from review of patient's records. Since patients were reviewed in at roughly 6 monthly intervals at both centres accurate documentation of exact time of loss of ambulation was not always possible and would often rely on parent recalling this information. Again the information on GC dose was estimated

from patient records. There were some differences in the annual bone heath assessments. Most notably bone density measures were acquired on different bone densitometers. However, the machine differences were minimised by using Z-scores from a robust reference dataset [22]. There were also differences in the imaging type from which vertebral fractures were assessed annually but again these differences were minimised by reviewing the images and consensus reporting by 2 investigators experienced in vertebral morphometry (JEA & NJC). It is feasible that the assessment of bone density may be affected by differences in adiposity. Experimental work with phantoms has shown that increasing adiposity increases the detected bone area and consequently results in artificially lower bone density values [31]. However, these measurement errors have not been quantified in children and as such it is difficult to assess the true impact they have on our different treatment groups with statistically different levels of adiposity. Consequently, further work on the relationship between lumbar spine BMAD and vertebral fracture is required as this study was not powered to statistically evaluate changes in bone density.

5. Conclusion

In summary, boys on a daily GC regimen tend to remain ambulant longer but at the cost of significantly more VFs, greater adiposity and markedly diminished growth. In contrast, boys on the intermittent GC regimen had fewer fractures but tended to lose ambulation earlier. In both groups, LS BMAD was a poor predictor of VFs. The high prevalence of VF, and the limited value of DXA to predict VF, suggests the need for VF screening as part of the boys routine bone health assessments. Finally, the decline in volumetric bone density as measured by peripheral QCT may be a more sensitive measure of bone loss and vertebral fracture risk.

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Table 1 Baseline characteristics of the DMD boys in each GC regimen (Mean (SD))

	DAILY Steroid Regime (n = 25)	INTERMITTENT Steroid Regime (n = 25)	Difference p value
Age (years)	8.1 (2.3)	8.5 (2.7)	NS
Height SDS	-1.4 (0.9)	-0.8 (1.0)	0.04
Weight SDS	0.3 (0.9)	0.1 (1.1)	NS
BMI SDS	1.5 (0.9)	0.8 (1.0)	0.01
Age started glucocorticoids	6.0 (1.9)	6.5 (1.8)	NS
Duration of Steroids (years)	2.1 (1.6)	2.0 (2.2)	NS
Cumulative glucocorticoid dose (mg)	11278 (7248)	6397 (7543)	0.02
Lumbar Spine BMAD (g/cm^3) Z-Score	-0.6 (0.7)	-0.3 (1.0)	NS
Total Body Less Head BMD (g/cm^2) Z-Score	-2.3 (1.2)	-1.8 (0.8)	NS
4% Distal Radius Total Density (g/cm^3) Z-Score	-0.5 (1.5)	-0.8 (1.2)	NS
4% Distal Radius Trabecular Density (g/cm^3) Z-Score	-1.1 (1.5)	-1.3 (1.2)	NS
Number of boys with long bone fractures	1	3	
Number of boys with vertebral fractures	1	0	

Note:

Abbreviation: BMAD Bone Mineral Apparent Density

Table 2 Mean (SD) Bone density Z-scores of DMD boys on DAILY and INTERMITTENT steroid regimen (excluding 6 boys who started bisphosphonate treatment due to symptomatic vertebral fractures)

	DAILY Steroid Regime			**INTERMITTENT Steroid Regime**		
Z-Scores	Baseline n = 19	Year 1 n = 19	Year 2 n = 19	Baseline n = 25	Year 1 n = 25	Year 2 n = 25
Lumbar Spine BMAD	-0.6 (0.7)	-0.6 (0.7)	-0.4 (0.9)	-0.3 (1.0)	-0.1 (1.0)	-0.1 (1.1)
*Total Body Less Head BMD	-2.3 (1.2)	-2.4 (1.5)	-3.1 (1.3)	-1.8 (0.8)	-1.9 (0.9)	-2.0 (0.9)
*4% Distal Radius Total Density	-0.2 (1.3)	-1.0 (1.4)	-1.0 (1.4)	-0.8 (1.2)	-1.2 (1.0)	-1.4 (1.2)
*4% Distal Radius Trabecular Density	-0.9 (1.4)	-1.2 (1.0)	-1.9 (1.3)	-1.3 (1.2)	-1.5 (1.1)	-1.8 (1.2)

Note:

Abbreviation: BMAD Bone Mineral Apparent Density

* Significant change over time ($p<0.05$) but no significant interaction between GC regime and bone density over time.

Table 3 Mean (SD) Bone density Z-scores of boys with and without vertebral fractures at the end of the study (excluding 6 boys who started bisphosphonate treatment due to symptomatic vertebral fractures)

	No Vertebral Fractures			**Vertebral Fractures**		
Z-Scores	Baseline (n = 38)	Year 1 (n = 38)	Year 2 (n = 38)	Baseline (n = 6)	Year 1 (n = 6)	Year 2 (n = 6)
Lumbar Spine BMAD	-0.4 (0.8)	-0.3 (0.8)	-0.2 (0.9)	-0.4 (1.4)	-0.6 (1.4)	-0.7 (1.6)
Total Body Less Head BMD	-1.8 (0.9)	-2.0 (1.1)	-2.3 (1.2)	-2.7 (1.0)	-2.7 (0.9)	-3.2 (1.1)
*4% Distal Radius Total Density	-0.5 (1.3)	-0.9 (0.7)	-1.1 (1.3)	-1.2 (0.7)	-2.2 (0.7)	-2.3 (0.6)
*4% Distal Radius Trabecular Density	-1.0 (1.2)	-1.3 (1.1)	-1.7 (1.2)	-2.1 (1.2)	-2.0 (0.8)	-2.6 (1.3)

Note:

Abbreviation: BMAD Bone Mineral Apparent Density

*Significantly different at baseline and significant change over time ($p<0.05$) but no significant interaction between fracture and bone density over time.

435 **Figure Legends**

436

437 **Figure 1** Changes in ambulation over time (a). There was a trend for more boys on the DAILY
438 GC regime (20%) (Black boxes) to remain ambulant after two years than boys on the INTERMITTENT
439 (40%) (Grey boxes). Occurrences of vertebral fracture (b). Significantly more vertebral fractures were
440 reported in boys on the DAILY regime (40%) (Black boxes) than the INTERMITTENT regime (8%)
441 (Grey boxes) (χ^2; $p = 0.008$).

442

443 **Figure 2** Bone density differences between the vertebral fracture group (n= 6, black circles)
444 and non-vertebral fracture group (n= 38 grey squares). (a) Demonstrates no significant difference in
445 LSBMAD Z-scores between groups (n= 38). In contrast, (b) demonstrates the reduction in baseline
446 TotBMD Z-scores at the 4% distal radius for DMD boys who suffered a vertebral fracture and their
447 greater drop in TotBMD Z-scores over time, as measured by pQCT.
448